ANNEXES

Annex 1
Research topics

Research topics RT-projects

	TOPICS	Maximum duration (months)	Maximum grant
	Animal health		
1	New mapping of sensitive natural areas in Belgium and dynamics of exposure of industrial and hobby poultry farms for the risk of exposure to low and highly pathogenic influenza viruses (FLUCART)	24	€ 200,000
2	Validation of gE tank milk testing for maintaining IBR-free status (MilkIBR)	24	€ 200,000
	Plant health		
3	Newly emerging risks of pests for plants and plant products in Belgium (EMPHYPEST)	30	€ 250,000
4	Evolution of potato cyst nematode populations in Belgium and control strategies (GLOBEVO)	36	€ 200,000
	Food safety		
5	Study on the concentration of nicotinic acid in fresh meat, minced meat, meat preparations and meat products (NICOMEAT)	12	€ 100,000
6	Pathogenic *Bacillus cereus* in foodstuffs: origin, growth and production of cereulide (BAGROCEP)	24	€ 200,000
7	Research on PFAS contamination in the food chain (PFASFORWARD)	48	€ 400,000

Research topics RI-projects: plant health - Euphresco

	TOPICS	Maximum duration (months)	Maximum grant[i]
	PPlant health		
2022-A-410	Frass-based detection of wood boring pests	24-36	€ 100,000
2022-C-412	The use of heat- (incl. hot water) treatments to eliminate plant pests (HETREAT)	24-36	€ 100,000
2022-F-415	*Meloidogyne enterolobii* – Survival under temperate climate conditions and distribution	24-36	€ 100,000
2022-A-418	Influence of incubation of wood samples on detection of pine wood nematode (PWN)	12-24	€ 100,000

[i] The FPS Health foresees **€ 200,000** to the transnational call. A maximum of € 100,000 can be requested per topic.

1. New mapping of sensitive natural areas in Belgium and dynamics of exposure of industrial and hobby poultry farms for the risk of exposure to low and highly pathogenic influenza viruses (FLUCART)

Background

In recent years, Europe has experienced an upsurge in cases of highly pathogenic avian influenza (HPAI) in its industrial (commercial) and hobby (non-commercial) poultry farms, mainly caused by H5Nx clade 2.3.4.4 type viruses. This upsurge is concomitant with a worldwide panzootic of H5Nx HPAIv cases. HPAIv epidemics in the poultry sector are closely linked to the migration of wild birds, which continually import new strains of HPAIv into the wildlife, where they can establish themselves in an endemic state and from there cause spill-over into the poultry sector. Currently, only biosecurity measures (confinement /housing, protective nets, etc.) can be put in place to prevent such introduction into the poultry sector. These measures usually apply at national level but have severe economic and animal welfare consequences.

Areas of facilitated contact between wild and captive poultry species have been identified in the past. However, they can be reviewed to update and gain a more accurate view of the current risk areas, allowing the risk manager to target and maximise the efficiency of its actions.

Research questions

- Determine in advance and on the basis of the hydrological profile, the risk areas in Belgium for contact between wild (non-captive) birds on the one hand and (captive) poultry species and other ornamental birds on the other hand.
- Develop a pertinent sampling strategy in these areas that is representative in time and space for poultry species (commercial and non-commercial sectors) as well as wild birds.
  From the avian samples collected, establish the genetic profile and the pathogenicity of the strains circulating in these areas and compare them with the most recent profiles for the whole country.
- Depending on the results obtained, re-edit as precisely as possible (minimum NUTS 3, or even up to NUTS 4/LAU 1) the current map of sensitive natural areas where there is a risk of contact between poultry from either the commercial or non-commercial sector and wild birds.

Maximum budget: **€ 200,000**

Maximum duration: **24 months**

2. Validation of gE bulk milk testing for maintaining IBR-free status (MilkIBR)

Context

Belgium has a national eradication programme for IBR (Infectious Bovine Rhinotracheitis) in place since January 2007. For the first 5 years, this was a voluntary programme. Since 5 January 2012, the programme has been mandatory in order to achieve an IBR-free status for Belgium. The Belgian IBR eradication programme was officially approved by the European Commission on 8 October 2014. Belgium acquires the 'Article 9' status and could therefore impose conditions on intra-community trade.

Since 21 April 2021, the new European Animal Health Law[i] (AHL) has been in force. Article 85 of the Delegated Regulation 2020/689[2] supplementing this AHL provides that Member States which have an officially approved eradication programme in place at the time of entry into force of the AHL may maintain this status for a maximum period of 6 years, provided that the national programme is adapted to the new rules of the AHL.

Furthermore, this Delegated Regulation 2020/689 sets out the new rules on IBR.

Annex III, Section 4 of this Regulation 2020/689 establishes the diagnostic methods for granting and maintaining IBR-free status. This annex allows the use of (bulk) milk testing only in non-vaccinated cattle, notwithstanding the presence of DIVA-vaccinated cattle on a farm with free status. Moreover, the limit for the pooled samples is set quite strictly.

In Belgium the matrix bulk milk has been allowed on predominantly milk-supplying farms since 2020 **to maintain the IBR-free status**, whereby a gE ELISA is used as test method. This was done on the basis of findings in an earlier FPS-funded project (RF 12/6263 IBRDIA[3]). On predominantly milk-supplying farms, periodic bulk milk testing is a cost- and labour-saving alternative to (annual) serological screening for maintaining a free status. In addition, bulk milk testing allows early detection of possible contamination: unlike an annual sampling, bulk milk samples are taken at a minimum of 6 times throughout the year, as opposed to an annual blood sampling.

In Belgium, there were approximately 6.680 predominantly milk-supplying farms in 2021. Today, some 2.600 companies in Belgium use bulk milk to maintain the IBR-free status.

The aim of this project is to investigate whether the current testing methods included in the Belgian eradication programme, as part of **maintaining the IBR-free status**, are at least equivalent to the testing methods included within the AHL (annual serological screening/sampling). As part of the eradication programme, the majority of Belgian cattle farms have been vaccinated in recent years. There is a need for DIVA diagnostics; the gE-ELISA test on bulk milk is a useful tool for predominantly milk-supplying farms in this context.

[1] Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on communicable animal diseases and amending and repealing certain acts in the field of animal health ('Anial Health Law')

[2] Delegated Regulation 2020/689 supplementing Regulation 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programmes and disease-free status for certain listed diseases and emerging diseases.

[3] RF 12/6263 IBRDIA – Support of the IBR (Infectious Bovine Rhinotracheitis) control programme by optimization of diagnostic detection methods

The government aims to be able to submit a dossier to the European Commission by 2027 with an application to obtain IBR-free status at Belgian level. In order to substantiate this dossier, it should be demonstrated that the IBR gE ELISA performed on bulk milk samples, as used in the Belgian eradication programme, is a testing regime at least equivalent to those currently included in the AHL.

Furthermore, the AHL imposes a maximum number of cattle per bulk milk sample, which in many cases excludes the bulk milk matrix on professional dairy farms with more than 100 cattle per tank. Here, too, there is a need to consider how this can be translated to the Belgian context.

Research questions

1. Is periodic sampling via the matrix bulk milk (minimum 6 to 9 times a year) and testing with the IBR gE ELISA on farms where DIVA-vaccinated cattle are present equivalent to serological monitoring based on individual samples taken during an annual random sampling in the context of maintaining the IBR-free status? For the serological sample, it is accepted that this method has a detection limit of 10%. For the bulk milk tests, it must be demonstrated that one (weakly) positive animal can be detected in the pooled sample.
2. Is the limitation of 100 milk samples within a bulk milk sample an obstacle for maintaining the IBR-free status on the current milk-supplying farms in Belgium? Is the sensitivity of current diagnostic methods* sufficient when applied to larger farms (more cattle in a tank)?
    - *ELISA for the detection of total antibodies specific to BoHV-1 or antibodies targeting BoHV-1 glycoprotein B*
    - *ELISA for the detection of antibodies targeting BoHV glycoprotein E*

Maximum budget: **€ 200,000**

Maximum duration: **24 months**

3. Newly emerging risks of pests for plants and plant products in Belgium (EMPHYPEST)

Context

Under the new plant health legislation, the list of EU quarantine organisms is set out in Annex II of the Implementing Regulation (EU) 2019/2072[1]. In case of a finding, measures need to be imposed to eradicate this organism, unless it is a quarantine organism known to occur in limited numbers in the EU and for which containment is allowed at a European level (Annex II, part B).

In addition to pest organisms already listed, monitoring and protection against new risks was also stepped up:

- A list of high-risk plants, plant products and other materials has been established[2]. These are prohibited for import into the EU until a dossier on the matter has been submitted to and evaluated by the EFSA (European Food Safety Agency) and further assessed at an EU level.
- Various information channels (scientific literature, import interception reports, media publications, outbreak data in the EU or third countries, etc.) are monitored by the EPPO (European and Mediterranean Plant Protection Organisation), the EFSA, the European Commission and the Member States in order to more rapidly identify information on changing situations or findings in the EU and in third countries.

It is essential that, after identification of a new organism presenting a risk, there is monitoring and evaluation of the phytosanitary risk and of the possible need for action in the EU. An important element here is the occurrence or non-occurrence and the degree of spread of the pest in the EU (see the conditions for qualifying a pest as a quarantine organism laid down in Article 3 of Regulation 2016/2031).

However, in a great number of cases, there is a lack of sufficiently targeted, research-based information on the current phytosanitary situation of these organisms in the EU (in this case, Belgium).

The issue also arises for organisms considered as quarantine by third countries and for which Belgium cannot provide sufficient information regarding the pest status to meet the international obligations in this regard.

Conducting a survey is the most reliable way to determine or verify such status (ISPM 8, revised version for 2021[3]). In addition, for some of these organisms, there is a need for diagnostic method development or further knowledge-building.

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[1] Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 laying down uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and of the Council, as regards protective measures against plant pests, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019

[2] Commission Implementing Regulation (EU) 2018/2019 of 18 December 2018 establishing a provisional list of high-risk plants, plant products or other objects within the meaning of Article 42 of Regulation (EU) 2016/2031 and a list of plants for which a phytosanitary certificate is not required for introduction into the Union within the meaning of Article 73 of that Regulation

[3] IPPC Secretariat. 2021. Determination of pest status in an area. International Standard for Phytosanitary Measures No 8. Rome. FAO on behalf of the Secretariat of the International Plant Protection Convention.

Given that the new plant health legislation places a stronger emphasis on prevention, timely awareness-raising among scientists, operators and the general public is important. Research into the presence of new high-risk pests can make an important contribution to this.

Research questions

Based on a number of ongoing policy-regulatory initiatives for which additional research data is needed, a selection of pests was drawn up. The research proposal should include a justified choice from this list of pests, taking into account the interrelationship of the selected pests (e.g. taxonomic group, host plant, sector/crop), the monitoring of at least two and, if possible, three growing seasons and the necessary budget. At least six of the proposed organisms should be included.

- What is the phytosanitary status of selected pests in Belgium?
- What methodology and monitoring plan is appropriate to underpin this, taking into account biology, geographical distribution, host plants, introduction and establishment potential?
- What additional research is needed (such as the finalisation/validation of diagnostic methods and research into the susceptibility of host plants,…)?

List of pests:

- *Chionaspis pinifoliae*
- *Crisicoccus pini*
- *Toumeyella parvicornis*
- *Phenacoccus solenopsis*
- *Garella musculana*
- *Pochazia shantungensis*
- *Colletotrichum* sp. with problem as recently identified in the EFSA assessments: *C. fructicola, C. plurivorum, C. siamense*, etc.
- *Phytophthora* species: *Phytophthora pluvialis*: *Phytophthora nemorosa, Phytophthora hibernalis, Phytophthora austocedri, Phytophthora lateralis*

Maximum budget: **€ 250,000**

Maximum duration: **30 months**

4. Evolution of potato cyst nematode populations in Belgium and control strategies (GLOBEVO)

Background

The control strategy for potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) that was applied since 2010 (implementation of Council Directive 2007/33/EC of 11 June 2007 on the control of potato cyst nematodes and repealing Directive 69/465/EEC) has been repealed on 31/12/2021 and replaced by a regulation to align it with the new plant health regulation (Regulation (EU) 2016/2031).

Since 2010, the presence of *Globodera* spp. has been monitored throughout the territory of Belgium and before planting, compulsory analyses are carried out on all plots for the production of seed potatoes or plants for planting. More than 500 plots have been officially declared infested and are subject to official control measures (prohibition on growing plants for planting, possibility of producing ware potatoes provided that resistant varieties are used, etc.). In addition to these official controls, growers are able to carry out their own self-checking analyses before planting potatoes and can receive assistance from research organisations to develop their own control methods. Current results show a dominance of *G. rostochiensis* infestations but also an increasing presence of *Globodera pallida*, especially in areas where farm-saved seed potatoes are grown. In addition, populations of potato cyst nematodes with increased pathogenicity have been reported by Germany and the Netherlands.

During this period, several research projects were funded in the context of potato cyst nematode control, including RF 07/6188 GLOBODERA[1], RT 08/11 COBEGLO[2] and RF 12/6264 DIVERGENCY[3].

Research questions

1. How have the populations of *Globodera* spp. evolved in the different Belgian potato production areas in terms of species (distribution of the different species), pathogenicity (virulence, bypassing of varietal resistances, reproduction rate, impact of the pest on production, etc.)? What are the factors that determine this evolution?
2. What control strategies should be put in place to prevent the development of new, more pathogenic populations and ensure their effective management where they appear?
    a. Are the current monitoring and analysis methods effective in detecting the emergence of populations with increased pathogenicity? If not, what methods of monitoring and rapid pathogenicity testing should be put in place?
    b. Are the current control methods effective in relation to the emergence of populations with increased pathogenicity? If not, what control measures should be put in place at national, local or plot level (e.g. rotations, rational use of resistant varieties, tare land treatment/management)?

[1] RF 07/6188 GLOBODERA - Situation and risk analysis of the spread of the potato cyst nematode (*Globodera spp.)* in the potato sector
[2] RT 08/11 COBEGLO - Cost-benefit analysis of the control of *Globodera rostochiensis* and *G. pallida*
[3] RF 12/6264 DEVIRGENCY - Detection of low cyst densities, knowledge of virulence groups and generation time of *Globodera spp.* as management tools for the potato cyst nematode

c. What recommendations could be made to operators to prevent and reduce these infestations as much as possible (e.g. self-checking with rapid on-site diagnostic tools)?

The results of the available international research will have to be taken into account to answer these questions.

Maximum budget: **€ 200,000**

Maximum duration: **36 months**

5. Study on the concentration of nicotinic acid in fresh meat, minced meat, meat preparations and meat products (NICOMEAT)

Background

Vitamin B3 ($C_6H_5NO_2$) is a water-soluble vitamin that consists of two molecules: nicotinic acid and its amide, nicotinamide. It is naturally present in a certain ratio of nicotinic acid to nicotinamide in many foodstuffs, including meat and meat products, fish, yeast and mushrooms. Vitamin B3 is also biosynthesised in the liver from tryptophan, an essential amino acid (SHC Advice n° 9285, 2016[1]).

Nicotinamide is much less toxic than nicotinic acid. Acute toxicity has been observed following excessive consumption of nicotinic acid over a short period of time. The adverse effects associated with nicotinic acid are due to the release of histamine (EFSA, 2014[2]). Given their different levels of toxicity, the Superior Health Council (SHC, 2016[1]) has established maximum total intakes for consumers (ranging from 2-10 mg/d for nicotinic acid versus 150-900 mg/d for nicotinamide, depending on age).

According to the Codex Alimentarius (1995), nicotinic acid has the property of being a red colour fixing agent in meat and meat products. The European Union does not allow the use of nicotinic acid as a food additive.

However, the FASFC has been confronted with cases of high levels of nicotinic acid in fresh meat, minced meat, meat preparations and meat products (minced meat, sausages, hamburgers, etc.), and suspects a fraudulent addition. Concentrations were such that consumers were admitted to hospital, suffering from red skin blotches, itching and skin irritation. An investigation by the National Investigation Unit (NIU) identified the operator who was illegally supplying nicotinic acid to its customers (butchers, caterers and fishmonger).

The addition of nicotinic acid to meat can lead to food poisoning in consumers as a result of two phenomena. Firstly, hospitalisation may be required due to the acute toxic effects of the ingestion of this compound. Secondly, while nicotinic acid is found to have a real effect on preserving the colour of fresh meat, its fraudulent addition may mislead the consumer and expose him to the consumption of meat that may be microbiologically unsafe (Advice SciCom 12-2021[3]).

In order to protect consumers, the FASFC wants to be able to detect fraudulent additions of nicotinic acid. A request has been made to the Scientific Committee (SciCom) to propose an action limit for nicotinic acid in fresh meat, minced meat, meat preparations and meat products, based on which fraudulent addition can be identified (Advice SciCom 12-2021).

Unfortunately, there is very little (reliable) data available that gives an indication of the natural concentration of nicotinic acid, as well as the ratio of nicotinic acid to nicotinamide, in meat. These data are needed to differentiate what may be a normally expected nicotinic acid content from excessive (added) and potentially dangerous content. A knowledge of the nicotinic acid/nicotinamide ratio naturally expected in meat should make it possible to detect any addition of nicotinic acid that would directly impact the value of this ratio.

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[1] HGR NR. 9285 – Voedingsaanbevelingen voor België – 2016; CSS n° 9285 - recommandations nutritionnelles pour la Belgique – 2016

[2] EFSA Scientific Opinion on Dietary Reference Values for niacin, EFSA Journal 2014;12(7):3759. https://doi.org/10.2903/j.efsa.2014.3759

[3] Advice 12-2021 of the Scientific Committee established at the FASFC concerning action limits for nicotinic acid in fresh meat, minced meat, meat preparations and processed meat

The Scientific Committee has therefore recommended acquiring additional data in the framework of a research project.

Research questions

It is possible that nicotinic acid is added fraudulently under the guise of adding vitamin B3. It is not inconceivable that an enzyme is wittingly added to convert nicotinamide to nicotinic acid. To distinguish possible cases of fraud, it is imperative to know the natural concentrations of and the ratio between nicotinamide and nicotinic acid. It would be useful for the FASFC to have data on nicotinic acid and nicotinamide in samples of fresh meat taken directly at the slaughterhouse as well as on samples of minced meat, meat preparations and meat products after production and/or processing and/or storage. The study should focus on the measured

levels of nicotinic acid and nicotinamide, the natural presence levels that can be deduced, the ratio between the two compounds and their stability over time, so that natural presence can be distinguished from intentional addition considered as fraud.

Concretely, the research plan would be the following:

- Carry out a representative sampling of the production and processing chain for red meat (from the slaughterhouse to the butchery counter). Taking into account the shelf life and the breed of origin of the animal from which the meat comes as variables for this sampling.
- Determine the baselines for nicotinic acid and nicotinamide in order to infer the ratios and concentrations that may be naturally expected.

Maximum budget: € 100,000

Maximum duration: 12 months

6. Pathogenic *Bacillus cereus* in foodstuffs: origin, growth and production of cereulide (BAGROCEP)

Context

In the project RT 17/05 SPECENZYM *'Study on the purity of food enzymes for the development of general purity criteria for food enzymes'*, funded by the FPS Health, 17 of the 39 examined food enzyme preparations tested positive (presence in 25 g) for (suspected) *Bacillus cereus*. Many strains were also found to possess genes encoding emetic and/or enterotoxins. Because of this high prevalence, it is appropriate to evaluate the public health risk of *B. cereus* in enzymes and enzyme preparations.

As the action limit for *B. cereus* proposed by the Scientific Committee established at the Federal Agency for the Safety of the Food Chain is 10^5 cfu/g (SciCom Advice 23-2018)[1], a quantitative determination is necessary to assess this risk. One should take into account that conditions (e.g. moisture content, time and temperature) for optimal enzyme activity are usually also favourable for microbial growth. Irreversible inactivation of the food enzyme via heating does not guarantee the elimination of the toxins formed (*in casu* cereulide). Moreover, the use of food enzymes is very broad. They can be added at any stage of the food chain in almost all food categories. In addition, not all foods in which food enzymes are used are heated (e.g. dairy products with food enzymes). More information on food enzyme applications can be found on the FPS Health website[2] and the European Commission website[3].

At the European level, there is currently no microbiological criterion for *B. cereus*, with the exception of a process hygiene criterion for dried infant formula and dried dietary foods for special medical purposes intended for infants below six months of age[4].

The uncertainties concerning the food safety risk of *B. cereus* in food enzymes and food enzyme preparations that were revealed through the SPECENZYM project raise the question of whether an analogous risk exists in other food ingredients and the foodstuffs in which they are used. Further research is recommended to study in depth this risk in relevant foodstuffs and food ingredients.

A recent publication by Ellouze *et al*. (2021) [5] presents results of a study of the food groups that could be susceptible to cereulide production.

Currently, there is insufficient knowledge regarding the contamination of ingredients with pathogenic cereulide-producing *Bacillus cereus* and the formation of the toxin in the food chain.

In the project RT 09/02 BACEREUS *'Study of toxin production by Bacillus cereus, characterisation and detection of the strains responsible for food poisoning', B. cereus* has already been studied in more detail. However, in this study the focus was on the enterotoxin produced by this bacterium in the gut.

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[1] Advice 23-2018 of the Scientific Committee of the FASFC concerning estimation of the risk to the consumer of *Bacillus cereus* in food

[2] https://www.health.belgium.be/nl/voeding/specifieke-toegevoegde-stoffen/voedingsenzymen/wat-een-enzyme

[3] https://ec.europa.eu/food/safety/food-improvement-agents/enzymes_en

[4] Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs

[5] Ellouze M, Buss Da Silva N, Rouzeau-Szynalski K, Coisne L, Cantergiani F and Baranyi J (2021) Modeling Bacillus cereus Growth and Cereulide Formation in Cereal-, Dairy-, Meat-, Vegetable-Based Food and Culture Medium. Front. Microbiol. 12:639546. https://doi.org/10.3389/fmicb.2021.639546

Research objectives

1. To determine, based on literature review and expertise, the scope of foodstuffs and food ingredients and processes within which uncertainties exist regarding the food safety risk of *Bacillus cereus* through bacterial growth and cereulide production.

   To develop choices for the further course of the project. Certain food enzymes and food enzyme preparations (whether in dehydrated form or not) should be included in this scope, as well as two other case studies.

2. Quantitative determination of pathogenic *B. cereus* in food enzymes, food enzyme preparations and relevant food ingredients (as determined in research question 1).

   Analysis of toxin genes for cereulide.

   Identification of the contamination source(s) and pathway(s) through which *B. cereus* enters food enzymes and food enzyme preparations and other relevant foodstuffs and their ingredients.

3. Determination of the potential growth of *B. cereus* and potential cereulide production in the relevant food ingredients (as determined in research question 1 and developed further in research question 2).

   Determination of factors that may influence the bacterial growth and the cereulide production through the food chain. For food enzyme(s) (preparations), the following steps should be taken into account:
   a. Storage of food enzyme preparations;
   b. Use/action of the food enzyme in the foodstuff (where cases should be chosen with favourable conditions for microbial growth);
   c. Further processing, storage and use of the foodstuff

4. To estimate the food safety risk of pathogenic *B. cereus* with regard to cereulide-producing strains.

Maximum budget: € 200,000

Maximum duration: 24 months

7. Research on PFAS contamination in the food chain (PFASFORWARD)

Context

In July 2020, the EFSA set a new Tolerable Weekly Intake (TWI) of 4.4 nanograms per kg body weight per week for the sum of four perfluoroalkyl substances (PFASs), specifically perfluorooctane sulfonic acid (PFOS)[1], perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS). The EFSA also pointed out that this TWI is exceeded for a portion of the European population, which is of concern.

PFAS are man-made chemicals used in a variety of applications (e.g., textiles, household products, firefighting, automotive, food processing, construction, electronics). They are toxic substances belonging to the group of persistent organic pollutants (POPs): they remain present in the environment for a very long time (several years or decades) and bioaccumulate (Advice SciCom 22-2020[2]).

One route by which PFAS can enter the food chain is through the use of contaminated fertilising products. For example, PFAS have recently been found in compost and sewage sludge. On 16 July 2022, the new Fertilising Products Regulation (EU) 2019/1009[3] will come into effect. This allows recycled and organic materials to be used for fertilisation. Recycled and organic nutrients are also widely used in Belgium for the production of fertilising products.

Aside from fertilising products, PFASs can also be found in irrigation water. Through the use of contaminated irrigation water, fruit and vegetables might become contaminated.

Food-producing animals can be contaminated with PFAS through contaminated feed, drinking water and/or the environment (e.g. soil ingested by free-range chickens). The results of several transfer studies can be found in scientific literature. The Scientific Committee has studied the transfer of PFAS from animal feed to eggs, farm animal meat and cow's milk (Rapid opinion SciCom 10-2021[4]).
FASFC analyses show that farm animals with outdoor free range are most at risk of PFAS contamination.

The ongoing project RF 21/6350 FLUOREX "*Exposure assessment of perfluoroalkyl substances as follow-up on the concerns raised in the recent opinion of EFSA*", funded by the FPS Health, is investigating PFAS exposure via foodstuffs. For the purpose of performing an exposure assessment for Belgian consumers, sensitive analyses are being performed on foodstuffs as they can be purchased by consumers, including fishery products, meat, eggs, milk, fruit and vegetables, cereals, drinks and processed and compound foods including baby food. These focus on the four PFAS included in the EFSA's TWI. Currently the methods are extended to other relevant PFASs. The number of samples remains limited by the available resources.
So far, negotiations for the setting of legal maximum levels are only ongoing for certain foodstuffs of animal origin. A European monitoring recommendation is also under development[5], which recommends a very extensive list of PFASs be measured in an extensive

[1] EFSA CONTAM Panel, Scientific Opinion on the risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 2020;18(9):6223, 391 pp. https://www.efsa.europa.eu/en/efsajournal/pub/6223
[2] SciCom Advice 22-2020 - The evaluation of the FASFC analysis programme for exogenous contaminants: B. Persistent organic pollutants (POPs)
[3] Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003
[4] SciCom Rapid opinion 10-2021 - Perfluoroalkyl substances (PFAS) in food of animal and vegetable origin
[5] Draft Commission recommendation on the monitoring of perfluoroalkyl substances in food (SANTE 2021-10010) – *available upon request*

set of foodstuffs. This monitoring recommendation aims to prepare future policy; it may then allow to ask the EFSA for an update of the exposure assessment and the risk assessment and to extend the standardisation.
There are already indications that different PFASs are found in foodstuffs of plant origin than in foodstuffs of animal origin. A different set of PFASs has also been standardised in drinking water[6] than the four PFAS in the EFSAs TWI, and other PFASs are already found in human blood[7].

When standards apply to foodstuffs, there are implicitly also standards for derived and compound foods (article 2 of Regulation (EC) N° 1881/2006[8]). The question arises whether it would be possible to (roughly) estimate the PFAS level, say, for baked goods with many eggs in the recipe, for liver pate based on the liver concentration, or for black tripe based on blood measurement data.
Little is known about the parts of foodstuffs wherein PFAS are found. Such knowledge is important for policy and for establishing risk management measures, as well as for estimating the impact of standards. Imagine that PFAS in potatoes could be removed by peeling the potatoes: this is relevant knowledge. Can PFAS in fish be removed by filleting the fish? What is the difference in PFAS concentration and PFAS profile between, for example, liver, bacon with rind and pork tenderloin for the same pig carcass, or the difference in contamination between beef liver, tongue and steak?
PFAS are assumed to be stable in foodstuff during processing in the industry, but which PFAS end up in which fractions of foodstuffs and to what extent? In which milling fraction of grain can contamination be found? What is the fate of PFAS in the pressing of fruit juice or oil, the production of cheese and whey powder, surimi, gelatine or food additives, such as emulsifiers? More research is needed for the use of process factors in application of article 2 of Regulation 1881/2006 to the maximum levels set for basic foodstuffs, or for the setting of specific maximum levels for specific processed products.

Research questions

Study of perfluoroalkyl substances in food:

1. Analysis of an extensive list of PFAS in a representative number of (potentially) relevant foodstuffs in line with the European monitoring recommendation, with low limits of quantification.

   In addition to the four PFAS included in the EFSA's TWI, other PFAS should be included based on an informed choice of PFAS found in foodstuffs and/or in human blood and/or from the 20 PFAS standardised in drinking water. This should include analysis of potatoes, mushrooms and other vegetables, fruits, food for infants and toddlers and beverages (non-alcoholic beverages, wine and beer). Which PFAS appear to be most relevant to which foods?

   It should be possible to transfer the data to the EFSA database within the timeframe set out in the European recommendation, i.e. before 1 October 2026.

[6] Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption
[7] FAVV persbericht 22/12/2021 Milieuverontreiniging PFAS: onderzoek naar achtergrondwaarden in Vlaamse landbouwproducten afgerond; FASFC Communiqué de presse 22/12/2021 Contamination environnementale aux PFAS : monitoring de fond dans les produits agricoles flamands achevé
[8] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

2. Research into the behaviour and distribution of PFAS within a foodstuff in order to identify in which parts the PFAS are concentrated, with possible differences in PFAS profiles (proportion of individual PFAS to total PFAS present) between parts of a foodstuff based on the physicochemical properties of the individual PFAS.
   Examples: What is the proportion of PFAS in the peels of fruit or potatoes? What differences are there between parts of the same carcass? What can be found in the skin of fish compared to the fish fillet?
3. Research into the fate of PFAS in food processing; examination of process factors.
   Examples: In which fraction do PFAS end up in the production of processed fishery products, egg products (such as egg yolk), dairy products (e.g. production of cheese and whey powder), fruit juice, oil pressing, grain milling fractions...
   Estimates of *worst case* process factors for priority compound foodstuffs, e.g. liver products and baked goods rich in eggs.
4. Research into relevant sources and contamination pathways of PFAS in the food chain through literature review, experiments or simulations, with the aim of better understanding how it is that foodstuffs are contaminated as they are.
   Maximum use should be made of available data on the presence of PFAS in irrigation water, compost, sewage sludge, feed materials, animal feeds, well water and soil, among others.

   Additional experiments could include:
   a. *If data are not yet available:* Analysis of PFAS in compost and sewage sludge used for fertilisation in the food chain, and in irrigation water used in the food chain.
   b. *If possible:* Research into the transfer of PFAS in fertilising products and in irrigation water to foodstuffs of vegetable origin.
   c. Analysis of a representative number of relevant feed materials and animal feeds.
   d. *Possibly:* Analysis of well water used as drinking water for food producing animals.

Experimental transfer studies from animal feed and drinking water to foodstuffs of animal origin are beyond the scope of this call; a number of cases have already been described.

PFASFORWARD is not an exposure study, as this is the subject of the ongoing project RF 21/6350 FLUOREX.

The research must be complementary to existing or ongoing research. Researchers should make deliberate choices, focused on the relevance to consumer exposure and the risk that may be associated with it, and focused on the relevance for risk management such as standard setting, control and prevention. The data must be reported in an appropriate format so that it can be included in the EFSA's database to enable its use for policy.

If the submitters are involved in the European Partnership for the Assessment of Risks from Chemicals (PARC), the possibilities for use of the results within its work programme should be indicated.

Maximum budget: € 400,000

Maximum duration: 48 months

2022-A-410 Frass-based detection of wood boring pests

Short description

Attacks of many quarantine wood-boring beetles, such as the Emerald ash borer (EAB, *Agrilus planipennis*), can go on unnoticed for some time due to larvae living inside the host trees. Not until larval feeding has gone on for some time where trees start to develop symptoms or the population grows to be large enough where adults hatching are plentiful enough to increase the likelihood of detection may an outbreak be noticed.

However, pest eradication is more likely to succeed the earlier that pest detection is accomplished. A common challenge is that likelihood of detection is lower when the population densities are low, as can be expected early n following an introduction. This can be further hampered by lack of sensitive trapping strategies. During detection surveys and also while managing outbreaks it can be beneficial to employ several different types of survey och diagnostic methods in order to increase likelihood of finding the pest. A complementary approach to trapping is surveillance of trees in order to detect exit holes and/or larval tunnels and galleries that are indicative of the presence of a pest. In the case where pest exit holes or larval tunnels/galleries are not very characteristic and larvae are no longer present in the tree other traces of the pest such as frass can frequently be encountered. In such scenarios frass analysis that allows identification of the pest depositing the frass would be a valuable decision support for risk managers and NPPOs.

Description of the end product

Survey of current frass diagnostic methods available, diagnostic protocols for sample collection and diagnostics of pests using frass

Provisional other funders

- Swedish Board of Agriculture, Sweden (contact: Mr. Kristof Capieau, Kristof.Capieau@jordbruksverket.se)
- Department for Environment Food and Rural Affairs, United Kingdom (contact: Ms Jasmine Burr-Hersey, Jasmine.Burr-Hersey@defra.gov.uk)
- Science and Advice for Scottish Agriculture, United Kingdom (contact: Mr David Kenyon, david.kenyon@sasa.gov.scot)
- Ministry of Agriculture Forestry and Food, Slovenia (contact: Ms Erika Oresek, erika.oresek@gov.si)
- Federal Ministry of Food and Agriculture, Germany (contact: Ms Silke Steinmöller, silke.steinmoeller@julius-kuehn.de)
- Federal Office for Agriculture, Switzerland (Contact: Mr Andreas von Felten, andreas.vonfelten@blw.admin.ch)
- Department of Agriculture, Water and the Environment, Australia (contact: Mr Con Goletsos, PHSgovenancegroups@agriculture.gov.au)

Provisional project duration

24-36 months

2022-C-412	The use of heat- (incl. hot water) treatments to eliminate plant pests (HETREAT)

Short description

Hot water treatments can be used on Vitis against *Viteus vitifoliae* (EPPO Standard PM 10/16), against Grapevine flavescence dorée phytoplasma (EPPO Standard PM 10/18) and considered efficient against *X. fastidiosa* (EFSA, 2015). The question was raised whether other time-temperature combinations should be used to reduce plant mortality. It would be useful to compare how these treatments are done in practice in different countries. Heat- treatments can also be used on strawberry plants to control *Aphelenchoides besseyi* and *Aphelenchoides fragariae* (EPPO Standard PM 10/19). Hot air treatments have been shown to eliminate *Verticillium dahliae* from Olive plants (Morello et al., 2016). The use of these treatments should be investigated for other pest/host combinations (e.g. on olive plants against *X. fastidiosa*). These treatments could be used for the exportation or circulation of plant reproductive material from infected areas, or in the context of certification schemes.

Description of the end product

Validation of heat-treatments as phytosanitary measures

Provisional other funders

- Ministry for Primary Industries, New Zealand (contact: Ms Aurélie Castinel, Aurelie.Castinel@mpi.govt.nz)
- French Agency for Food, Environmental and Occupational Health & Safety, France (contact: Ms Géraldine Anthoine, geraldine.anthoine@anses.fr;jean-emmanuel.gerbault@anses.fr)
- Department of Agriculture Food and the Marine, Ireland (contact: Ms Maria Laura Destefanis, Maria.Destefanis@agriculture.gov.ie; Mr Conor MacGee, Conor.McGee@agriculture.gov.ie; Mr Pat Fitzgerald, pat@beotanics.com)
- Department of Agriculture, Water and the Environment, Australia (contact: Mr Con Goletsos, PHSgovenancegroups@agriculture.gov.au)

Provisional project duration

24-36 months

2022-F-415 ***Meloidogyne enterolobii*** **– Survival under temperate climate conditions and distribution within Europe**

Short description

The polyphagous tropical root-knot nematode *Meloidogyne enterolobii* is recently added to the list of EU quarantine pests. *Meloidogyne enterolobii* is known to be present in several (sub)tropical countries in North, Central and South America, Africa and Asia, where most epidemiological studies have been carried out. However, this species was also detected on roses (plants for planting) originating from China (see EPPO RS 2008/107), suggesting that it can also survive more temperate conditions. It is of great importance to generate within this project knowledge about the survival and duration of the life cycle of *M. enterolobii* in order to assess its potential impact on agri- and horticulture in Europe's temperate climate zone. Moreover, recent reports of *M. enterolobii* in Portugal and the ongoing outbreak in glasshouses in Switzerland demonstrate that this tropical root-knot nematode has the potential to enter and establish in (the warmer parts of) the EU and in glasshouses throughout the EU. This emphasises the importance to assess the distribution in Europe by conducting reliable and sensitive surveys and (import) inspections to prevent introduction and further spread of this highly damaging species. By sharing knowledge, comparing sampling and identification methods, a harmonised and consistent approach for performing surveys for *M. enterolobii* can be achieved.

Description of the end product

Knowledge about the survival and duration of the life cycle of *M. enterolobii* and on its current distribution

Provisional other funders

- National Plant Protection Organization, Netherlands Food and Consumer Products Safety Authority, Netherlands (contact: Mr Martijn Schenk, M.Schenk1@nvwa.nl)
- Federal Ministry of Agriculture, Regions and Tourism, Austria (contact: Ms Sylvia Bluemel, sylvia.bluemel@ages.at)
- Department of Agriculture Food and the Marine, Ireland (contact: Ms Maria Laura Destefanis, Maria.Destefanis@agriculture.gov.ie; Mr Pat Fitzgerald, pat@beotanics.com)
- Ministry of Agriculture Forestry and Food, Slovenia (contact: Ms Erika Oresek, erika.oresek@gov.si)
- Norwegian Food Safety Authority, Norway (contact: Ms Hanne Skomedal, hanne.skomedal@nibio.no)
- Centre for Researhc in Agricultural Genomics, Spain (contact: Ms Marta Sánchez, marta.sanchez@cragenomica.es)
- Eskisehir Osmangazi University, Turkey (contact: Mr Refik Bozbuğa, refikbozbuga@gmail.com; Mr Mustafa İmren, m.imren37@gmail.com)

Provisional project duration

24-36 months

2022-A-418	**Influence of incubation of wood samples on detection of pine wood nematode (PWN)**

Short description

Bursaphelenchus xylophilus is presumably introduced from North-America into Asia via contaminated wood in the early 20th century. So far, *B. xylophilus* has a limited distribution in Europe, having only a few findings under eradication in Spain and a restricted distribution in Portugal. By surveillance of pine stands and inspection of imported wood, bark and wood packaging material one can reliably monitor the pest and prevent further spread in the EU. Reliable detection methods are available, but depend on incubation of the wood material prior to the extraction of nematodes. According to the current guidelines, the diagnostic process including incubation can take up to 4 weeks. Pending the result of the analysis, the consignment must be kept under supervision of the authorities. In this project, the aim is to investigate the effect of the incubation period on detection of PWN, especially for low-level infestations in dry wood as wood packaging material.

Description of the end product

Optimised detection of PWN

Provisional other funders

- National Plant Protection Organization, Netherlands Food and Consumer Products Safety Authority, Netherlands (contact: Mr Martijn Schenk, M.Schenk1@nvwa.nl)
- All Russian Plant Quarantine Center, Russian Federation (contact: Mr Yuri Schneider, yury.shneyder@mail.ru)
- Ministry of Agriculture and Forestry, Finland (contact: Ms Marja Savonmaki, marja.savonmaki@gov.fi)
- Forestry Commission, United Kingdom (contact: Ms Joan Webber, joan.webber@forestresearch.gov.uk)
- Department of Agriculture Food and the Marine, Ireland (contact: Ms Maria Laura Destefanis, Maria.Destefanis@agriculture.gov.ie)
- National Institute for Agricultural and Veterinarian Research, Portugal (contact: Ms Leonor Cruz, leonor.cruz@iniav.pt)
- Federal Ministry of Food and Agriculture, Germany (contact: Ms Silke Steinmöller, silke.steinmoeller@julius-kuehn.de)
- Ministry of Agriculture Forestry and Food, Slovenia (contact: Ms Erika Oresek, erika.oresek@gov.si)
- Council for Agricultural Research and Economics, Italy (contact: Mr Sauro Simoni, sauro.simoni@crea.gov.it)
- Federal Office for Agriculture, Switzerland (Contact: Mr Andreas von Felten, andreas.vonfelten@blw.admin.ch)
- Swedish Board of Agriculture, Sweden (contact: Mr. Kristof Capieau, Kristof.Capieau@jordbruksverket.se)
- Centre for Research in Agricultural Genomics, Spain (contact: Ms Marta Sánchez, marta.sanchez@cragenomica.es)

Provisional project duration

12-24 months